Toxicity of Propylene Glycol 1,2-Dinitrate in Experimental Animals[1]

R. A. JONES, J. A. STRICKLAND, AND J. SIEGEL

U.S. Navy Toxicology Unit, National Naval Medical Center, Bethesda, Maryland 20014

Received August 12, 1971

Toxicity of Propylene Glycol 1,2-Dinitrate in Animals. JONES, R. A., STRICKLAND, J. A., and SIEGEL, J. (1972). *Toxicol. Appl. Pharmacol.* **22**, 128–137. The po toxicity of propylene glycol 1,2-dinitrate (PGDN) in rats and the ocular and dermal irritation effects of PGDN on rabbits were determined. Rats, guinea pigs, rabbits, squirrel monkeys and dogs were exposed to the vapors of PGDN in acute, repeated and continuous inhalation exposures. Physiological and biochemical changes noted were anemia, pigment deposition in various organs, fatty changes in the liver, methemoglobin formation, and greatly increased serum and urinary inorganic nitrates. The effect of PGDN on the avoidance behavior of trained rhesus monkeys was also studied.

The characteristic toxicologic effects reported for the aliphatic nitrates include vasodilation, lowered blood pressure, headaches and methemoglobin formation. Of more serious import are the sudden deaths due to circulatory failure reported by Carmichael and Lieben (1963) among workers exposed chronically to mixtures of nitroglycerine and ethylene glycol dinitrate (EGDN). These unexplained deaths have been attributed to the EGDN component of the mixture.

Only a limited amount of work has been done on propylene glycol 1,2-dinitrate (PGDN), a compound closely related to EGDN. Kylin *et al.* (1964) made a comparative ip toxicity study of PGDN, EGDN and nitroglycerine and found that the latter was 4 times more toxic to mice than EGDN or PGDN; the difference between EGDN and PGDN was small. Clark and Litchfield (1969) determined the LD50 for Alderly Park rats to be 1190 mg/kg PGDN. They concluded from po, sc and percutaneous comparative toxicity studies that there was little difference in the metabolism or pharmacologic properties of PGDN and EGDN when tested in rats, mice and cats.

Although untoward effects of PGDN which occur in ammunition workers usually result from skin absorption and/or inhalation, no toxicologic information is available on inhalation exposures. A series of experiments, including long-term animal inhalation studies, was therefore conducted with this compound.

[1] The opinions expressed herein are those of the authors and do not necessarily reflect the view of the Navy Department or the naval service at large. The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council, Washington, D.C.

10

PRELIMINARY STUDIES

Acute po, ocular, dermal and inhalation screening studies, as well as subacute dermal screening studies, were conducted prior to long-term inhalation tests. Because pure PGDN is unstable, the material used throughout these studies was essentially PGDN with a physiologically and chemically inert diluent of low volatility added. All the concentrations reported herein are based on the PGDN fraction of the mixture.

Oral toxicity. Single doses of the material were administered by po intubation to 36 male NMRI: (LE) Long-Evans rats at PGDN dose levels ranging from 76 to 3320 mg/kg. At the higher PGDN levels (1100–3320 mg/kg) there was a reduced response to external stimuli, prostration, mild convulsions and death. Initial signs were noted within 15 min; the severity of the response increased up to the time of death. At the lower PGDN doses (76–760 mg/kg) the signs noted were greatly delayed and not as severe as those seen at higher doses. The LD50, calculated by the method of Litchfield and Wilcoxon (1949), was 860 mg/kg with 95% confidence limits of 556 to 1325 mg/kg PGDN. Po intubation of the diluent at the highest dose level (1500 mg/kg) did not result in any toxic signs or death.

Ocular and primary skin irritation. Male New Zealand albino rabbits were utilized in skin and ocular irritation studies conducted by the method of Draize (1959). No effects were seen after 24 and 72 hr in the primary skin irritation tests. In the ocular study, 0.1 ml of the sample was instilled into 1 eye of the rabbits. Although there was no immediate reaction, redness of the conjunctiva was noted 5 min after instillation. The iris and cornea were not involved. The redness gradually abated and disappeared entirely within 24 hr.

Subacute dermal toxicity. A 20-day subacute skin absorption study was conducted by a modification of the method of Draize (1959). Male New Zealand albino rabbits were held in a restraining rack inside a ventilated hood during daily application of the PGDN mixture. After approximately 2 hr the excess material was wiped off with cotton gauze, and the rabbits were returned to their individual cages. Additional groups were treated in a similar manner with olive oil and diluent to serve as controls. The doses applied and the results obtained are shown in Table 1. All survivors were observed for 9 days or longer after the last application.

The rabbits in the 1 g/kg group showed minor skin irritation evidenced by slight erythema and roughening of the skin; this cleared up 5 days after the twentieth application. The animals did not show any other toxic signs and had an average weight gain of 11% during the application period. After the third application to the 2 g/kg group the rabbits appeared weak and slightly cyanotic, and demonstrated rapid, shallow breathing. One rabbit died after the sixth application. The remaining animals showed steady improvement and, with the exception of a slight wrinkling and scaling of the skin in the area of application, appeared normal and showed a weight gain of 15% on day 20. In the 4 g/kg group all rabbits became weak and cyanotic after the second application; 13 of 14 animals were dead after the fifth application. At autopsy, the internal organs were found to be dark, blue-gray in color, and the urinary bladder was markedly distended. Terminal body weights indicated an average loss of 11% over the application period of 4–5 days, whereas control rabbits showed an average weight gain of 4% after 10 days.

The total and differential leucocyte counts remained within normal limits at all 3 dose levels. As shown in Table 1, there was a lowering of the hemoglobin and hematocrit values after 4 applications of PGDN; the decreases were most marked in the highest dose group. In the 4 g/kg group, methemoglobin values determined by the method of Evelyn and Malloy (1938) rose from a base of less than 0.2 to 34.5% at death. Urinary nitrate (M. H. Litchfield, 1967) excreted by animals in the 4 g/kg dose group accounted for approximately 7% of the PGDN applied.

TABLE 1

MORTALITY, WEIGHT CHANGES AND SELECTED HEMATOLOGIC VALUES FOR RABBITS IN A SUBACUTE DERMAL TOXICITY EVALUATION OF PGDN

Material	Dose (g/kg)	No. of doses	*n*	No. died	Body weight (g) Initial	Body weight (g) % Change	Hemoglobin (g/100 ml)	Hematocrit (%)
PGDN + diluent	1	0	6	0	2429	0	13.7	41
		4		0		—	11.1[a]	38
		20		0		+11	13.1	44
	2	0	5	0	2677	0	12.9	38
		4		0		—	9.5[a]	34[b]
		20		1		+15	12.6	44
	4	0	14	0	2600	0	13.1	41
		5		13		−11	9.4[a]	29[a]
Olive oil + diluent	4	0	14	0	2892	0	13.3	40
		4		0		—	12.1	39
		10		0		+4	—	—

[a] $P < 0.01$.
[b] $0.01 < P < 0.05$.

Biochemical assays of liver and kidney oxygen uptake rates, serum protein electrophoretic fractions and specific activities of alkaline phosphatase, glutamic–oxaloacetic transaminase, glutamic–pyruvic transaminase, isocitric dehydrogenase and lactic dehydrogenase failed to reveal any consistent significant alteration which could be related to PGDN. Histopathologic examination of sections of heart, lungs, liver, spleen, kidneys, brain, spinal cord and skin indicated degenerative changes of the liver, renal vacuolar change of the proximal convoluted tubular epithelium and granular degeneration of the myocardial fibers in some of the animals. The remaining organs appeared normal.

Short-term inhalation studies. Six rats were exposed for 4 hr to a mist of the material containing PGDN (1350 mg/m^3) in a 30-l glass dynamic exposure chamber described by Leach (1963). No mortality occurred, and no toxic signs were noted during the exposure or within the 14 days postexposure observation period; the mean methemoglobin value immediately post exposure was 23.5%. One rabbit was exposed 23 hr per day at a mean concentration of 240 mg/m^3. On day 4 the rabbit became cyanotic and had a methemoglobin value of 18.2%. The exposure was continued and the animal died 6 hr later. The methemoglobin value at death had increased to 32.8%. A squirrel monkey was likewise exposed to the PGDN vapors at a concentration of 415 mg/m^3; it died on day 3 and had a methemoglobin value of 40.2%.

LONG-TERM INHALATION STUDIES

Repeated Inhalation Study

A repeated inhalation exposure was conducted at 65 mg/m^3 with 8 male NMRI:O (SD) Sprague-Dawley derived rats. The animals were housed in a 30-l chamber and exposed 7 hr/day, 5 days/wk, for a total of 30 exposures. Air at 1 l/min was passed over 3 ml of the test material contained in a Schwartz absorption tube maintained at 30 C. The contaminated air was then mixed with 3 l/min of dilution air just prior to entering the chamber. The contaminant concentration was monitored by a modified diphenylamine analysis[2] for nitrates. After each daily run the rats were returned to their individual cages for food and water.

No toxic effects or mortalities were noted, and all rats gained weight at a normal rate. Hematologic values determined before and after the exposure did not differ significantly. Four of the rats were autopsied immediately; the remaining 4 were autopsied after a 2-wk observation period. No abnormal findings were noted. Histopathologic examination of sections of heart, lungs, liver, spleen and kidneys did not show any changes that could be attributed to the exposure.

Continuous Inhalation Studies

Three studies were conducted in which 4 species of animals were exposed continuously, 24 hr/day, for 90 days. Fifteen NMRI:O(SD) Sprague-Dawley derived rats of both sexes, 15 NMRI:(ASH) Princeton or FTD: Hartley derived guinea pigs of both sexes, 9 male squirrel monkeys (*Saimiri sciureus*) and 2 male AKC registered beagle dogs were used in each study. A control chamber containing equal numbers of animals, treated the same as the test animals except for contaminant, was run concurrently with each experiment. The rats and guinea pigs were placed in individual cages, and the monkeys were housed 3 to a cage within the exposure chamber. All animals were provided the dry chow appropriate for the species; in addition, dogs received meat-based canned food, guinea pigs received water-soaked lettuce and monkeys were given oranges and hard-boiled eggs. Lettuce was the only source of water for the guinea pigs, while the other animals had water available at all times.

The inhalation exposure chambers used have been described previously by Fultyn (1961). Air flow through the chambers was maintained at 1 m^3/min, the temperature at 77 ± 2 F and the relative humidity at approximately 50%. The test material was placed in 2 4-l flasks and heated in constant-temperature water baths maintained at 50-55 C. The liquid was constantly agitated with magnetic stirrers, and the vapors were swept out of the flasks with predried air at 9-24 l/min; 3/8-in. stainless steel

[2] Chamber air was drawn through a midget impinger containing 10% acetone in water at 0.5 l/min for 10 min. The volume was then brought up to 20 ml with the sampling medium; 1 ml of the sample was placed in a stoppered graduate. Three working standards were treated similarly.

Working standards. A primary standard was prepared by diluting 1 ml PGDN up to 500 ml with reagent grade acetone. Aliquots of the primary standard with enough additional acetone to make 10 ml were brought up to 100 ml with distilled water.

Diphenylamine reagent. Prepared according to Pfeilsticker (1932).

Analytical. To both the sample and the working standards were added 15 ml of the diphenylamine reagent. The color was allowed to develop for 30 min in a constant-temperature bath at 30-32 C. The samples and standards were read vs a reagent blank at 620 mμ on a spectrophotometer.

tubing was used to transport the vapors to the main dilution air stream immediately upstream of the chamber. All tubing was heated to prevent condensation. Temperature, rate of stirring and/or air flow through the 2 flasks was adjusted to achieve the desired chamber concentrations.

Total leukocyte, hemoglobin and microhematocrit determinations were made prior to and after exposure. During 1 experiment, 6 weekly determinations of methemoglobin and serum inorganic nitrate were made. At the termination of exposure the animals were sacrificed with an overdose of sodium pentobarbital and necropsied. Tissues were retained for histopathologic examination, and blood and tissue specimens were taken for biochemical determinations.

Results. Chemical analyses indicated that the 3 exposures were conducted at mean concentrations $\pm$ SD of 67 $\pm$ 8, 108 $\pm$ 11 and 236 $\pm$ 24 mg/m^3 PGDN; these values compared favorably with nominal input determined gravimetrically.

One monkey exposed to 236 mg/m^3 died on day 31 after exhibiting a weight loss of 18%; adult filarial parasites (*Dipetalonema*) in the abdominal cavity and a slight mottling of the liver were seen at autopsy. There were no other deaths or visible signs of toxicity noted in any of the other animals of the 3 exposures.

The rate of body weight gain in all exposures was essentially the same as that seen in controls. Postexposure hematologic values were all within normal limits for all species except for dogs exposed to 236 mg/m^3 PGDN; these animals showed decreases of 63 and 37% in their hemoglobin and hematocrit values, respectively. The methemoglobin and serum inorganic nitrate values obtained on animals exposed to 236 mg/m^3 PGDN are given in Table 2.

The only abnormality noted at necropsy was the presence of adult filarial parasites (*Dipetalonema*) in the abdominal cavities of a majority of the monkeys. Tissue sections of heart, lung, liver, spleen and kidney from all species, sections of brain, spinal cord and adrenals from dogs, and brain and spinal cord from monkeys, were examined for histopathologic changes.

The liver from both dogs in the 67 mg/m^3 exposure showed hemosiderin deposits in the sinusoids, bile canaliculi, Kupffer cells and liver plate cells. Similar pigment accumulations were seen in the cytoplasm of the epithelial cells lining the proximal convoluted tubules in the kidneys of the dogs and some of the rats. Fatty changes were noted in some of the guinea pig and rat livers.

In the 108 mg/m^3 exposure, guinea pigs consistently showed foci of pulmonary hemorrhage. Fatty changes similar to that seen in the 67 mg/m^3 exposure were also seen in the livers of dogs and monkeys. Monkeys had iron-positive granules present in the central areas of the liver and in some kidney sections. Liver sections from dogs revealed heavy iron-positive deposits in the sinusoids and Kupffer cells.

Heavy iron-positive deposits were also present in the liver, spleen and kidney sections of dogs and monkeys exposed to 236 mg/m^3. Hepatic iron-positive deposits were commonly associated with vacuolar change, mononuclear cell infiltrates and focal necrosis. Female rats showed focal necrosis of the liver and acute tubular necrosis of the kidney that appeared to be related to the test material; male rats appeared normal. Vacuolar changes noted in the liver of all guinea pigs and in 4 of 9 monkeys were also attributed to the exposures. No changes were noted in any of the other tissue sections examined from the 3 exposures. Control rats and guinea pigs were normal.

Squirrel monkeys exposed to 108 mg/m^3 and to 236 mg/m^3 PGDN had elevated serum urea nitrogen and decreased serum alkaline phosphatase levels (Table 3), indicating the possibility of kidney change in this species. Elevation in the bromosulfalein retention values in 1 dog exposed to 236 mg/m^3 was noted. Serum aspartate aminotransferase and liver alkaline phosphatase determinations were in close agreement with controls.

TABLE 2

METHEMOGLOBIN AND SERUM NITRATE VALUES OF ANIMALS EXPOSED CONTINUOUSLY TO PGDN (236 mg/m^3)

Species	Days of exposure	% Methemoglobin		Serum nitrate (μg/ml)	
		n	Mean ± SD	*n*	Mean ± SD
Monkeys	0	9	2.4 ± 0.8	9	12 ± 4
	7	1	10.7	2	100, 126
	14	2	17.3, 12.1	2	202, 386
	21	2	7.3, 5.6	2	153, 329
	28	2	5.4, 2.1	2	79, 80
	35	2	7.6, 4.3	2	190, 300
	42	2	3.4, 1.0	2	71, 82
Dogs	0	2	1.2, 1.7	2	2.4, 2.5
	7	1	19.4	1	140
	14	1	23.4	1	174
	21	1	20.2	1	118
	28	1	10.9	1	56
	35	1	20.4	1	139
	42	1	14.7	1	44
Rats	0	10	<0.2	—	—
	7	2	1.6, 1.7	—	—
	14	2	9.9, 12.8	—	—
	21	2	6.7, 4.2	—	—
	28	2	3.1, 5.5	—	—
	35	2	7.0, 8.0	—	—
	42	2	2.3, 7.3	—	—
Guinea pigs	0	10	0.2	—	—
	7	2	1.1, 1.5	—	—
	14	2	3.3, 2.2	—	—
	21	2	2.5, 1.5	—	—
	28	2	3.4, 6.6	—	—
	35	2	2.2, 5.3	—	—
	42	2	4.8, 9.3	—	—

Behavioral Studies on Rhesus Monkeys[3]

Prior to the start of inhalation exposures, an acute iv study was carried out using 12 untrained rhesus monkeys. A total of 6 dose levels using between 0.028 and 0.41 g/kg were administered to 2 monkeys each to determine the minimum dose at which toxic signs could be noted. Doses exceeding 0.041 g/kg produced emesis, retching, ptosis, ataxia and apnea of increasing degree and duration, with death occurring to the 2 monkeys at the 0.41 g/kg level.

[3] Hazelton Laboratories, Inc., Falls Church, Virginia, Contract No. N00014-67-C-0306.

TABLE 3

BIOCHEMICAL DATA IN ANIMALS EXPOSED CONTINUOUSLY TO PGDN BY INHALATION

Concentration PGDN (mg/m^3)	Serum alkaline phosphatase μmoles Substrate Degrade min/ml				Serum urea nitrogen (mg/100 ml)				Bromosulfalein retention (%)
	Rat	Guinea pig	Monkey	Dog	Rat	Guinea pig	Monkey	Dog	Dog
Control	23 ± 9	32 ± 7	153 ± 29	11 ± 4	16 ± 4	25 ± 6	28 ± 9	18 ± 5	5 ± 1
67 ± 8	21 ± 4	26 ± 4	—	—	19 ± 4	27 ± 3	30 ± 7	—	2,2
108 ± 11	23 ± 11	27 ± 5	90 ± 40[a]	12,12	18 ± 2	22 ± 3	64 ± 16[a]	18,19	7,5
236 ± 24	19 ± 10	13 ± 4[a]	58 ± 13[a]	15,25	13 ± 2	23 ± 4	67 ± 34[a]	12,14	4,25

[a] $P < 0.01$.

Four monkeys previously trained to perform in a visual discrimation test (VDT) described by Samuel *et al.* (1965) were then injected with 10% of the dose which produced a toxic effect, i.e., 0.004 g/kg, and after a 1-wk rest were injected with 0.007 g/kg. The animals were observed for pharmacotoxic signs 1 hr preinjection, immediately after injection and at 1-hr intervals for 3 hr postinjection while undergoing the VDT test. Minimal behavioral effects were noted in 1 animal at the lower dose group only.

On preliminary inhalation trials, monkeys exposed to 500–700 mg/m^3 for 6 hr exhibited signs of toxicity such as vomiting, pallor, cold extremities, semiconsciousness and clonic convulsions. All these toxic signs disappeared within 30 to 45 min after the animals were removed from the contaminated atmosphere.

After these preliminary studies, 3 monkeys trained in VDT or visual acuity threshold (VATT) tests (Coate, 1967) were exposed continuously for 90 days to 262 mg/m^3 PGDN; once each week however, the animals were removed from the chamber for a period of 2 hr for their respective behavioral tests. A 600-l chamber was used, and the dilution airflow was maintained at 100 l/min. A fourth trained control monkey was exposed to filtered room air under the same conditions.

During the 90-day test the only pharmacologic sign noted was mydriasis, which increased from slight to moderate; body weight changes were not significant. There were no changes in the avoidance behavioral pattern in the rhesus monkeys as indicated by the VDT and VATT tests.

DISCUSSION

Although po, ocular and primary skin irritation studies indicated that PGDN is relatively nontoxic to slightly toxic, the subacute percutaneous studies in rabbits showed that the material is absorbed through the skin. Evidence of this consisted of decreased hemoglobin and hematocrit, an increase in methemoglobin and urinary nitrate excretion, loss in body weight and death at the 4 g/kg level. In our study the urinary excretion accounted for about 7% of the applied PGDN. Clark and Litchfield (1969) were able to account for 56% of sc injected EGDN and PGDN as urinary nitrates. Hasegawa and Sato (1963) reported that EGDN injected into rabbits daily caused an increased nitrate excretion and they interpreted this as due to an increased formation of EGDN hydrolyzing enzyme.

Dogs exposed chronically to PGDN vapors at 236 mg/m^3 for 90 days also showed a marked decrease in hemoglobin and hematocrit levels. All 4 species exposed developed increased levels of methemoglobin; species differences were evident, with the dogs and monkeys being the most severely affected. This might reflect the species differences in methemoglobin reductase activity described by Stolk and Smith (1966). Serum inorganic nitrate levels were also greatly elevated in our study; Clark and Litchfield (1969) pointed out that inorganic nitrates are among the metabolic products of PGDN.

Pathologic findings in cases of chronic EGDN poisoning have been summarized by Patty (1963) as consisting of fatty changes in heart, liver and kidney, and pigment deposition in the liver and spleen characteristic of anemia. Rabbits used in our subacute dermal toxicity studies demonstrated granular degeneration of myocardial fibers. Chronic continuous inhalation exposure to PGDN in this study induced fatty changes in the liver of guinea pigs, dogs and monkeys; extensive hemosiderin deposition

was noted in the liver and kidney of the animals, probably as a result of increased destruction of erythrocytes. The deposition in the liver was approximately the same at all 3 levels; the deposition in the kidney appeared to be concentration-dependent.

In view of the apparent similarity of the toxicologic effects of EGDN and PGDN, one must consider the possibility that the unexplained deaths in workers attributed to EGDN, i.e., sudden circulatory collapse 24–72 hr after removal from exposure, may also occur with chronic exposure to PGDN. Urinary nitrate determinations have been used as an indication of the amount of EGDN absorbed in the blood stream of workers and would undoubtedly be useful for those exposed to PGDN.

Based in part on the above information and the fact that headaches have been reported[4] for man at levels of 1.2 to 4.8 mg PGDN/m^3, a tentative Confined Space Guideline (CSG) of zero was recommended by the Committee on Toxicology, NAS-NRC; this level was believed to be necessary to prevent a degradation of performance. A limit of 1.2 mg/m^3 was recommended for a 40-hr wk industrial-type exposure.

ACKNOWLEDGMENTS

The authors are grateful to L. J. Jenkins, Jr., R. A. Coon, J. Lyon, E. G. Fishman, D. E. Rector, E. Swab and D. Sanders for their assistance in various phases of this study. Dr. J. H. Leathem of Rutgers University, New Brunswick, New Jersey, and Dr. M. A. Jackson of Howard University, Washington, D.C. served as consultants in pathology. The constructive review and suggestions of R. C. Wands, Advisory Center on Toxicology, National Academy of Sciences—National Research Council is gratefully acknowledged.

REFERENCES

CARMICHAEL, P., and LIEBEN, J. (1963). Sudden death in explosive workers. *Arch. Environ. Health* 7, 424–439.

CLARK, D. G., and LITCHFIELD, M. H. (1969). The toxicity, metabolism, and pharmacologic properties of propylene glycol 1,2-dinitrate. *Tox. Appl. Pharmacol.* **15**, 175–184.

COATE, W. B. (1967). Monitoring visual activity in the rhesus monkey. In. *Proc. Contractors Conferences on Behavioral Sciences, 1965.* Edgewood Arsenal, Maryland, pp. 123–134.

DRAIZE, J. H. (1959). *Dermal Toxicity*, pp. 46–59. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. By the Staff of the Division of Pharmacology, Food and Drug Administration, Department of Health, Education and Welfare. Published by the Association of Food and Drug Officials of the United States. Business Office Bureau of Food and Drugs, Texas State Department of Health, Austin, Texas.

EVELYN, K. A., and MALLOY, H. T. (1938). Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. *J. Biol. Chem.* **126**, 655–662.

FULTYN, R. V. (1961). Contaminant generators for continuous exposure inhalation chambers. *Amer. Ind. Hyg. Ass. J.* **22**, 49–53.

HASEGAWA, H., and SATO, M. (1963). Experimental study on nitroglycol poisoning in rabbits. *J. Biochem. (Tokyo)* **54**, 58–64.

KYLIN, B., ENGLUND, A., EHRNER-SAMUEL, H., and YLLNER, S. (1964). A comparative study on the toxicology of nitroglycerine, nitroglycol and propylene glycol dinitrate. *Proc. 15th Int. Occup. Health Vienna* 3, 191–195.

LEACH, L. J. (1963). A laboratory test chamber for studying airborne material. *AEC Research and Development Report UR-629*, 1–12.

[4] Personal communication, Naval Ordnance Environmental Health Center, Cincinnati, Ohio.

LITCHFIELD, J. T., JR., and WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **96**, 99–113.

LITCHFIELD, M. H. (1967). The automated analysis of nitrite and nitrate in blood. *Analyst* **92**, 132–136.

PATTY, F. A (1963). *Industrial Hygiene and Toxicology*, 2nd ed., revised. Wiley, New York.

PFEILSTICKER, K. (1932). Colorimetric determination of nitrate with diphenylamine or diphenylbenzidene. *Z. Anal. Chem.* **89**, 1–8.

SAMUEL, G. K., KODOMA, J. K. and MENNEAR, J. H. (1965). Effects of scopolamine and atropine and their quarternized salts on avoidance behavior in the monkey. *Psychopharmacologia (Berlin)* **8**, 295–301.

STOLK, J. M., and SMITH, R. P. (1966). Species differences in methemoglobin reductase activity. *Biochem. Pharmacol.* **15**, 343–351.